INTRODUCTION: Currently there are no treatments available for osteoarthritis (OA). In order to establish new therapeutic strategies for the treatment of OA, a better understanding of the cellular and molecular changes during OA progression is required. The progressive ankylosis protein (ANK) is a transmembrane protein that transports intracellular pyrophosphate (PPi) to the extracellular milieu. Previous studies have shown that extracellular PPi and inorganic phosphate (Pi) resulting from PPi hydrolysis affect cartilage calcification as well as chondrocyte differentiation. Extracellular PPi has been shown to inhibit basic calcium phosphate (BCP) crystal formation. However, excessive extracellular PPi concentrations lead to the formation of calcium pyrophosphate (CPPD) crystals. Both types of crystals are found in human OA patients. In addition, several lines of evidence suggest that ANK via its cytoplasmic N-terminal and C-terminal domains interacts with other proteins and these interactions may modulate signaling pathways. Since ANK expression is low in normal healthy articular cartilage and its expression levels increase in OA pathology, we have determined the role of ANK in OA pathology.

METHODS: Mouse articular chondrocytes isolated from articular cartilage caps of 2-month-old ank/ank mice and wild type littermates were cultured as described. Because of a spontaneous mutation leading to a premature stop codon in the ank gene, ank/ank mice express a non-functional ANK protein, which does not insert into the plasma membrane and is degraded in the absence or presence of 10ng/ml interleukin-1 (IL-1). Post-traumatic OA was surgically induced in 4-week-old ank/ank and wild type mice using the transection of the medial collateral ligament and partial medial meniscectomy (PMMX) joint instability model. Human articular chondrocytes isolated from leftover tissue after knee replacement surgery were transfected with an expression vector and cultured in the absence or presence of interleukin-1 (IL-1) and zolendronate (10^4, 10^5, 10^6 M), a bisphosphonate and non-hydrolysable PPi analogue. The mRNA levels of articular chondrocyte markers (aggrecan and type II collagen), hypertrophic markers (alkaline phosphatase (APase), runx2, and type X collagen), and MMP-13 were determined by real time PCR. NF-kB activity was determined by the transfection of human chondrocytes with NF-kB-reporter plasmid. The mineralization of human chondrocytes in the absence or presence of PPi and levamisole (to prevent PPi hydrolysis) was determined using alizarin red S staining.

RESULTS: We first determined how the lack of ANK in ank/ank mice affects cartilage destruction and OA pathology. Proteoglycan loss and immunostaining for MMP-13 were markedly reduced in IL-1-treated ank/ank femoral head explants compared with IL-1-treated wild type explants. In addition, cartilage destruction and OA severity was markedly reduced in ank/ank mice 10 weeks after PMX surgery compared with wild type cells. Consequently, zolendronate in decreased MMP-13 mRNA levels and hypertrophic changes of articular chondrocytes. Consequently, zolendronate, a non-hydrolysable analogue of PPI, inhibited MMP-13 expression and hypertrophic changes of articular chondrocytes and increased aggrecan and type II collagen expression. In conclusion, ANK stimulates hypertrophic differentiation, mineralization, NF-kB activity, and MMP-13 expression in chondrocytes during OA pathology, whereas bisphosphonates as non-hydrolysable PPI analogues protect articular cartilage function and phenotype.

SIGNIFICANCE: The understanding of the mechanisms of how ANK affects articular chondrocyte function and phenotype during OA pathology may lead to the discovery of novel therapeutic targets for the treatment of OA.